GenTarget Inc

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Other Regents for Cell Immortalization

CAT#	Product Name	Amounts
LVP1134-RB	EBNA1 (RFP-Bsd) Lentivirus	**200ul,** (1 x 10^7 IFU/mL) in DMEM medium containing 10% FBS / 10 x (60 ug/m) polybrene
LVP1135-RB	EBNA2 (RFP-Bsd) Lentivirus	
LVP1136-RB	HpV16-E6 (RFP-Bsd) Lentivirus	
LVP1137-RB	E1A (RFP-Bsd) Lentivirus	
LVP1138-RB	HOXA9 (RFP-Bsd) Lentivirus	
LVP1139-RB	KRas_G12V (RFP-Bsd) Lentivirus	
LVP1140-RB	CDK4 (RFP-Bsd) Lentivirus	
LVP1141-RB	cMyc (RFP-Bsd) Lentivirus	
LVP1134-GP	EBNA1 (GFP-Puro) Lentivirus	
LVP1135-GP	EBNA2 (GFP-Puro) Lentivirus	
LVP1136-GP	HpV16-E6 (GFP-Puro) Lentivirus	
LVP1137-GP	E1A (GFP-Puro) Lentivirus	
LVP1138-GP	HOXA9 (GFP-Puro) Lentivirus	
LVP1139-GP	KRas_G12V (GFP-Puro) Lentivirus	
LVP1140-GP	CDK4 (GFP-Puro) Lentivirus	
LVP1141-GP	cMyc (GFP-Puro) Lentivirus	
LVP1134-RB-PBS	EBNA1 (RFP-Bsd) Lentivirus in **PBS**	**200ul,** (1 x 10^8 IFU/mL) in **PBS solution**
LVP1135-RB-PBS	EBNA2 (RFP-Bsd) Lentivirus in **PBS**	
LVP1136-RB-PBS	HpV16-E6 (RFP-Bsd) Lentivirus in **PBS**	
LVP1137-RB-PBS	E1A (RFP-Bsd) Lentivirus in **PBS**	
LVP1138-RB-PBS	HOXA9 (RFP-Bsd) Lentivirus in **PBS**	
LVP1139-RB-PBS	KRas_G12V (RFP-Bsd) Lentivirus in **PBS**	
LVP1140-RB-PBS	CDK4 (RFP-Bsd Lentivirus in **PBS**	
LVP1141-RB-PBS	cMyc (RFP-Bsd) Lentivirus in **PBS**	
LVP1134-GP-PBS	EBNA1 (GFP-Puro) Lentivirus in **PBS**	
LVP1135-GP-PBS	EBNA2 (GFP-Puro) Lentivirus in **PBS**	
LVP1136-GP-PBS	HpV16-E6 (GFP-Puro) Lentivirus in **PBS**	
LVP1137-GP-PBS	E1A (GFP-Puro o) Lentivirus in **PBS**	
LVP1138-GP-PBS	HOXA9 (GFP-Puro) Lentivirus in **PBS**	
LVP1139-GP-PBS	KRas_G12V (GFP-Puro) Lentivirus in **PBS**	
LVP1140-GP-PBS	CDK4 (GFP-Puro) Lentivirus in **PBS**	
LVP1141-GP-PBS	cMyc (GFP-Puro) Lentivirus in **PBS**	

Storage: <-70 °C, avoid repeat freeze/thaw cycles, stable for > 6 months.

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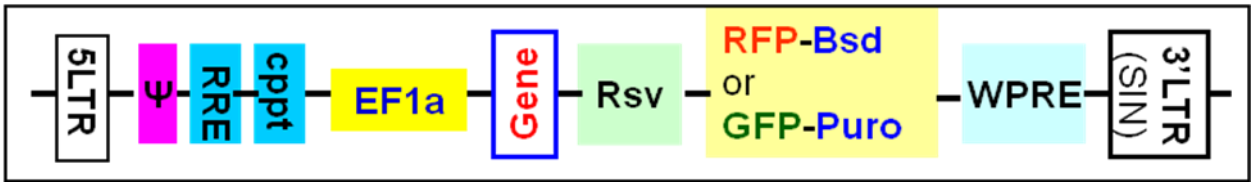
Product Description:

Normal cells will die after a few rounds of proliferation because of cellular senescence. There are a few methods to turn a primary cell to immortal so that the cells can undergo infinite cell divisions or large rounds of doubling in culture medium (Cell Immortalization).

The most widely use cell immortalization methods are to over-express SV40 large T-antigen or human TERT gene. In addition Sv40-T-antigen and hTERT gene, the other genes are also used for cell immortalization depends on cell types. The Epstein Barr Virus (EBV) genes (**EBNA1 and EBNA2**) were reported used for immortalize B and T lymphocytes, the HPV16 virus' **E6/E7** genes for keratinocytes, the Adenovirus type 5's **E1A** gene for primary rodent cells, the human HOX genes for various hematopoietic cells, including macrophages, hematopoietic progenitor cells, and myeloid progenitor cells, the human **CDK4** for human bronchial cells and myogenic cells, the human **KRas V12 mutant, cMyc** for a wide variety of cells and so on.

Gentarget provides the premade over-expression lentivirus products for those cell immortalization genes. Each gene was expressed under an enhanced enhanced **EF1a** promoter which is active in almost all cell types and less likely to be silenced during long-term culture. Each Lentivirus is featured with an antibiotic-fluorescent fusion dual maker, **GFP**-puromycin or **RFP**-Blasticidin. (see **vector map scheme** below).



VSV-G pseudotyped lentivirus are generated in 293T cell, and provided as 200 ul aliquots in two formats:

1) in DMEM medium containing 10% and 10x Polybrene (60 ug/ml) at titer of $1x10^7$ IFU/ml;
2) in PBS solution at titer of $1x10^8$ IFU/ml, for usage in serum-free cell culture;

For general questions about our ready-to-use particles, please see **FAQ for pre-made lentiviral particles** (.pdf) on our website. (http://www.gentarget.com/pdf/FAQ-Premade-Lentiviral-particles.pdf).

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Key features:

1. Each lentiviral particles contain an **antibiotic-fluorescent** fusion dual marker, used for selecting the transduced cells or generating stable cell lines by antibiotics selection or via fluorescent cell sorting.
2. The enhance **EF1a promoter** is active in all cell types and do not be silenced during long-term culture.
3. The lentivirus are ready and easy to use, simply add 50ul into one well culture in 24-well plate. No need any other reagents at application.

Transduction Protocols:

1. Transduction Protocol for Adhesive cells:

Note: Pre-made lentivirus is provided ready to use, so it can be simply added into your cell culture; the amount of virus to add depends on cell type. For quick transduction, add 50 µl of virus into each well of 24-well-plate where cell density is 50% to 75%. After 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection.

Day 0:

Seed cells in complete medium at the appropriate density and incubate overnight.

Note: at the time of transduction, cells should be 50%-75% confluent. For example, seed HeLa cells at 0.5 x 10^5/ml x 0.5ml in a well of a 24-well plate.

Day 1:

- Thaw the pre-made lentiviral stock at room temperature and add the appropriate amount of virus stock to obtain the desired MOI.
- Return cells to 37°C, CO_2 incubator. Do nothing.

  Note: Try to avoid freezing and thawing. If you do not use all of the virus at one time, you may re-freeze the virus at -80 °C for future use; virus titer will decrease by ~10% for each freeze/thaw cycle.

Day 3:

At 48hr~72hr (Depend upon cell type) after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava).

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Day 3 + (optional):

Sort transduced cells by FACS, or select by antibiotic killing. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line (refer to the pertinent literature on generation of stable cell lines).

2. Transduction Protocol for Suspension Cells:

Grow cells in complete suspension culture medium; use a shaking flask in a CO^2 incubator if required.

Measure cell density (not grow over 3 million/ml), measured viability should be > 90%. Dilute cells into 1 x 10^6 cell/ml in complete medium.

Day 1:

- Thaw lentiviral particles at room temperature.
- Add premade lentiviral particles into the diluted cells at a ratio of: 50 to 100 µl virus per 0.5 ml of cells (Note: depending on cell type, you may need to use more or less virus).
- Grow cells in a shaking flask in a CO2 incubator.

Day 2:

At 24 hours after transduction, add an equal amount of fresh medium containing. Continue growing cells in CO2 incubator.

Day 3+:

At 48 hour to 72 hours (Depend upon cell type) after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Pass cells into 0.5 million/ml density in completed medium containing the corresponding antibiotic (**Note:** amount of antibiotic depends on cell type. A killing curve must pre-established). Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

Note: Filter wavelength settings:

GFP filter: ~Ex450-490; ~Em525; RFP filter: ~Ex545; ~Em620;

Safety Precaution:

Gentarget lentiviral particles adapts must advanced lentiviral safety features (using the third generation vectors with self-inactivation SIN-3UTR), and the premade lentivirus is replication incompetent. However, please use extra caution when using

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lentiviral particles. Use the lentiviral particles in Bio-safety II cabinet. Wear glove all the time when handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

References:

1. Experimental Cell Research Volume 201, Issue 2, August 1992: 417-435
2. Genome Res. 2008 122 (3-4): 263–72.
3. Proc Natl Acad Sci U S A. 2003 Sep 16; 100(19): 10989–10994.
4. Semin Cancer Biol. 2001 Dec;11(6):423-34.
5. July 5, 2002.The Journal of Biological Chemistry 277, 24709-24716.
6. Mol. Cell. Biol. March 1988 vol. 8 no. 3 1036-1044
7. Methods in Enzymology Volume 439, 2008, Pages 1-13
8. Oncogene 2000 19, 608–616
9. Cancer Res. 2004 Dec 15;64(24):9027-34.
10. Cell. Volume 82, Issue 1, p29–36, 14 July 1995
11. Cancer Res 2005; 65: (6). March 15, 2005

Warranty:

This product is for research use only. It is warranted to meet its quality as described when used in accordance with its instructions. GenTarget disclaims any implied warranty of this product for particular application. In no event shall GenTarget be liable for any incidental or consequential damages in connection with the products. GenTarget's sole remedy for breach of this warranty should be, at GenTarget's option, to replace the products.

Attachment: GenTarget's pre-made lentivirus product categories.

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Repoter Lentivirus for all kinds of pathway screening assays
Cell Immortalization	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.
ImmunoOncology Research	Lentivirus products for immuno therapy research: CAR and TCR; Assay Cell Lines for T-cell targeted killing assay and other cell-based assays; over-expression lentivirus products for the immune response targets; Cell surface antigens (CDs); immune checkpoint / Receptors; CRISPR gene Repair and knock-IN lentivirus; CRISPR knockout lentivirus;
CAR-T, TCR Lentivirus	**CARs** Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; **TCRs**: MART-1/ NY-ESO1/

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	CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;
CRISPR Gene Editing	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease, the **dCas9**, gRNAs, **CRISPR** gene editing research
Epigenomic: CRISPRi and CRISPRa	"**dCas9-Protein**" fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
Cell-Specific Reporter	a set of reporter lentiviruses to express a luminescence or fluorescent reporter (firefly Luciferase, Renilla luciferase, RFP or GFP fluorescent marker) under a tissue specific promoter
Infectious Antigens	Llentivirus that express all kinds of infectious antigens with C-term 6His-tag.
Virus Like Particles (VLP)	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
Non-integrating LV	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
shRNA Knockdown	Knockdown verifeid and customized shRNA lentivirus for target knockdown,
microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
Anti-miNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Human and mouse ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla, Red-Luc and more,** with different antibiotic selection markers.
Fluorescent Markers	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.
Luminescent Imaging	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and

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	dynamic processes in living cells and organisms
Sub-cellular Imaging	Lentivirus contain a well-defined organelle targeting signal fusioned to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways.
Cytoskeleton Imaging	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
Unstable GFP	Lentivirus express the the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection
near-infrared RFP	The near-infrared Red fluorescent (niRFP) expression Lentiviurs provides the whole-body images with better contrast and brighter images
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.
CRE, Flp ColorSwtich	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or "FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the CRE or Flp recombination event in vivo.
SEAP Reporter	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
TetR Repressor	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF inducible system.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers
LacZ expression	Express different full length **β- galactosidase (lacZ)** with different selection markers
	Premade **negative control lentivirus with different**

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Negative control lentiviruses	**markers**: serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.
Ultra titer lentivirus	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.